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Physical and Biochemical Properties of Maize Hardness and Extrudates of Selected Hybrids

KYUNG-MIN LEE,[†] SCOTT R. BEAN,[‡] SAJID ALAVI,[§] TIMOTHY J. HERRMAN,*[,||] AND RALPH D. WANISKA[†]

Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas 77843; Grain Marketing and Production Research Center, Agricultural Research Service, U.S. Department of Agriculture, Manhattan, Kansas 66502; Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506; and Office of the Texas State Chemist, Texas A&M University, College Station, Texas 77841

Protein and starch determinants of maize kernel hardness and extruded products were characterized to better define the role of endosperm texture during extrusion. Maize physical properties were correlated with total proteins and zein subclasses ($p < 0.01$). The extrusion process significantly altered protein solubility and increased protein fragmentation as measured by RP-HPLC and size exclusion chromatography. Harder grits and extrudates demonstrated higher amylose content, lower degree of starch damage, and fragmentation at different screw speeds than softer grits and extrudates. Differences in extrudate expansion ratio, water absorption index, water solubility index, oil absorption capacity, and breaking stress between harder and softer hybrids were related to protein aggregation and fragmentation as well as starch damage and fragmentation.

KEYWORDS: Maize hardness; extrusion; zein; α-zeins; hydrophobicity; disulfide formation; starch fragmentation; extrudate property

INTRODUCTION

Maize (*Zea mays* L.) hardness is an important trait that influences end-use performance including milling and processing quality (*1*, *2*). Two biochemical components in maize, alcohol-soluble prolamins (zeins) and amylose in starch, have been described to be related to maize endosperm texture (*3*−*5*). Zeins in maize endosperm are generally divided into several subclasses (*5*): α-zeins (19 and 22 kDa), *β*-zeins (15 kDa), *γ*-zeins (16 and 27 kDa), and *δ*-zeins (10 kDa). The higher levels of α-zeins were found in horny endosperm more than in soft endosperm in maize (*6*). The *γ*-zeins are evenly distributed through the endosperm in quality protein maize, implying the possible involvement of this protein in maize kernel hardness (*5*). However, it is not clear which zein subclasses or members of multigene families play a more important role in determining maize hardness. Starch polymers are believed to influence maize hardness depending on relative amounts and the orientation of amylose and amylopectin molecules (*4*) and protein matrix surrounding starch granules (*2*). The starch synthesis is associated with the formation of alcohol-soluble proteins (*7*).

Extrusion processing greatly modifies macromolecular arrangement during the processing, resulting in unique products (*8*). The changes may occur in the physical and biochemical properties of protein molecules after extrusion, affecting protein solubility (*9*), breakage or formation of cross-linking, and nonspecific hydrophobic and electrostatic interactions between and within protein molecules (*10*, *11*). The extrusion process is unique in that starch gelatinization and melting occur at lower moisture content and high shear conditions. The amylose content, the amount and type of starch, the ratio of amylose and amylopectin, and the fragmentation of large amylopectin molecules (*3*, *4*, *12*) are factors that possibly affect the structural, mechanical, and functional properties of extrudates. Large amylopectin molecules are easily fragmented, decreasing the average molecular weight, whereas amylose molecules do not undergo significant change in molecular weight distribution (*12*). Starch fragmentation that occurs within an extruder and/or orifice is a function of extrusion processing conditions and the quality of raw material (*13*).

Maize with harder endosperm texture provides several advantages over that with softer endosperm texture during storage, transportation, handling, and processing. However, the inter-relationship of endosperm texture and biochemical components of maize on extrudate properties has been scarcely studied. In this study, maize hybrids from previously defined maize hybrid types (*14*) were evaluated for their physical hardness, protein and starch properties, and extrusion-processing performance. These physical and biochemical characterization of raw materials and extrudates can provide a more fundamental understanding of endosperm texture concerning the extrusion

* Corresponding author [telephone (979) 845-1121; fax (979) 845-1389; e-mail tjh@otsc.tamu.edu].
[†] Texas A&M University.
[‡] U.S. Department of Agriculture.
[§] Kansas State University.
[||] Office of the Texas State Chemist.

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Table 1. Means, Standard Deviations, and Ranges of Proximate Constituents and Hardness-Associated Properties for All Maize Hybrids from Which Samples for Extrusion Were Selected[a]

	protein (%)	oil (%)	starch (%)	test wt (kg/hL)	NIT density (g/cm³)	pycnometer density (g/cm³)	TADD (%)	time to grind in SHT (s)	absorbance at 860 nm
mean	8.54	3.49	60.58	73.5	1.286	1.278	37.70	21.6	2.703
SD[b]	0.58	0.34	0.75	1.1	0.019	0.020	5.57	3.1	0.136
range	7.00–9.90	2.55–4.35	58.80–62.6	67.6–78.6	1.235–1.320	1.202–1.322	17.07–51.30	13.5–32.8	2.419–3.233

[a] Range of moisture content: 13 ± 1%. [b] SD, standard deviation, $n = 248$.

process. As an outcome from this study, producers and processors will be better equipped to predict maize hybrid extrusion-processing performance and improve their selection criteria.

MATERIALS AND METHODS

Sample Selection. Maize hybrids grown at different locations in Kansas and Missouri were harvested manually during 2002 and 2003. Samples were shelled at an ambient temperature when they approached a moisture content below 16% and then stored in plastic bags at room temperature. Moisture contents of the samples used for the following tests were between 12 and 14%. Six maize hybrids were selected from statistical clusters that represent distinct physical and chemical hardness-associated properties, described by Lee et al. (*14*). These hybrids were classified into two major groups according to relative kernel hardness. Three hybrids (HARD1, HARD2, and HARD3) were considered to be harder and the remaining three hybrids (SOFT1, SOFT2, and SOFT3) softer.

Maize Quality and Hardness-Associated Properties. Maize kernel hardness was measured using several hardness-associated properties including test weight following the USDA's Federal Grain Inspection Service's protocol (*15*), specific density was measured by air comparison pycnometer (Quantachrome, Boynton Beach, FL) (*1*), abrasiveness was measured using the tangential abrasive dehulling device (TADD) (*16*), and the time required to grind kernels in the Stenvert micro-hammermill test (Glenmills model V with a 2 mm screen) run at 360 rpm was measured (*17*). Sample moisture, protein, oil, starch content, and density were measured using near-infrared transmittance (NIT) (Grainspec, Multispec Limited, Wheldrake, NY) that had been calibrated by the manufacturer and the Grain Quality Laboratory at Iowa State University against chemical methods. All measurements were duplicated and averaged. NIT spectra in a log[1/*T*] were obtained from whole maize kernels over the range of 850−1048 nm in 2 nm increments using an Infratec 1229 (Foss North America, Eden Prairie, MN) with a 30 mm path sample holder. Each sample's spectrum was the average of 10 individual scans. The collected spectra were further processed using software (WINSI II v 1.0, Foss NIRSystems, Infrasoft International, LLC., Silver Spring, MD). The absorbance at 860 nm was used as an indirect measurement of kernel hardness (*18*). The means, standard deviations (SD), and ranges of hardness-associated properties of all maize hybrids prior to selection of samples for extrusion are summarized in **Table 1**.

Dry-Milled Corn Grits Preparation. Dry milling was performed using an Allis laboratory mill using a long flow procedure that maximizes low-fat grit extraction (*19*). Maize samples were tempered to 18% moisture content for 45 min before milling. Dry-milled corn grits were sifted in a sieve shaker (model RX-86, W. S. Tyler, OH) and were prepared by mixing 0.35 fine (between the 420 and 841 μm sieves) and 0.65 coarse (between the 841 and 1191 μm sieves) grits to give similar particle sizes and distributions. The average particle size was calculated by applying the formula as described in Baker and Herrman (*20*). The average particle sizes of six maize grits are as follows: HARD1, 853 μm; HARD2, 854 μm; HARD3, 855 μm; SOFT1, 854 μm; SOFT2, 852 μm; and SOFT3, 855 μm. By using similar particle sizes and distributions among grit samples, the effect of particle size on extrudates could be eliminated because the average feed particle characteristics such as particle size, grit hardness, and grit surface of maize grits influence the expansion ratio and structural and mechanical properties of the final extrudates (*8*, *9*, *21*).

Extrusion Process. A Micro 18 twin-screw laboratory extruder (American Leistritz Extruder Corp., Somerville, NJ) was used and consisted of a six-barrel section with a 4.5 mm die diameter. The extruder barrel temperature setting and corotating screw configuration profiles are depicted in **Figure 1**. Grits were fed into the extruder barrel through a corotating screw feeder at a constant low 130 rpm screw speed, resulting in a feed rate of 20 g/min. Extruder die temperature and pressure were measured using a Dynisco pressure transducer equipped on the extruder. The mechanical energy input was calculated from the energy consumption of the electric motor and rpm values of the screw speed (N), using the formula

$$Q_{me}\ (\text{kJ/h}) = (L - L_0) \times P_e \times \left(\frac{N_a}{N_f}\right) \times 36$$

where Q_{me} is mechanical energy rate (kJ/h), L is motor load on the drive motor (%), L_0 is motor load (%) with no material in the barrel, P_e is the rated power of extruder drive motor (kW), N_a is the actual operating screw speed (rpm), and N_f is the full (rated) screw speed (rpm). Then, specific mechanical energy (kJ/kg) was expressed as the ratio of the mechanical energy rate divided by mass flow rate (kg/h).

On leaving the die, the extruded samples were cut into ≈30 cm lengths and dried to ≈7−8% moisture at 50 °C for 12 h. The average diameter of extrudate strands was calculated from 15 measurements using digital vernier calipers. The expansion ratio was calculated by dividing the squared average diameter of extrudates by the squared die diameter. The piece density was calculated from the average diameter and weight per unit length, assuming extrudate strands to have a cylindrical shape (*22*). Breaking stress was determined using a texture analyzer (Texture Technologies Corp., Scarsdale, NY). The long cylindrical extrudates were cut into 13 mm lengths with a sharp blade. The cylindrical 12 mm in diameter was used to compress the samples at a constant speed of 0.6 mm/s. A mean was calculated from five replicates. The force−distance curve was recorded. Breaking stress (rupture strength) was calculated as force divided by cross-section area of extrudate strands (*23*). For the measurements of water absorption index and water solubility index, the methods described by Anderson et al. (*24*) were used. Oil absorption capacity was determined as described by Lin and Humbert (*25*). For the determination of oil absorption capacity, a graduated centrifuge tube containing the ground extrudate and corn oil was held for 30 min and subsequently centrifuged at 1610*g* for 25 min to measure the free volume of oil. Moisture contents for grits and extrudates were determined using AACC method 44-15A (*26*).

Protein Characterization. For maize protein characterization, 100 mg of material was ground using an Udy mill (Udy Corp., Fort Collins, CO) equipped with a sieve of 0.25 mm diameter. Zeins (prolamins) for RP-HPLC were extracted with 60% *tert*-butyl alcohol (for chromatography of nonreduced grits and extrudates) and 60% *tert*-butyl alcohol containing 2% β-mercaptoethanol (β-ME) (for chromatography of reduced grits and extrudates) (*27*). RP-HPLC separations were conducted using an Agilent 1100 HPLC system equipped with a 4.6 mm × 250 mm Jupiter 300 Å C18 column (Phenomonex, Torrance, CA) with security guard columns (Phenomonex). For size exclusion chromatography, proteins were extracted as described in Batterman-Azcona and Hamaker (*28*). A Coomassie Plus Protein Assay Kit (Pierce, Perbio Science Co., Rockford, IL) was used for zein quantification. A standard curve was developed using commercial zein (0.2−8 mg/mL) to measure zein concentration in unknown samples. A linear response

Barrel temperature profile

Die	150°C	125°C	100°C	75°C	50°C	30°C (inlet)
←19mm→	←88 mm→	←88 mm→	←88 mm→	←88 mm→	←88 mm→	←88 mm→

Screw profile

11 (Die)	10	9	8	7	6	5	4	3	2	1 (Inlet)

Screw Element	Screw Element
[a] 1: SE GFA–2–30-90	7: SE GFA-2-10-30
2: SE GFA-2-30-30	[c] 8: KB 5-2-20-45 R
3: SE GFA-2-20-60	9: SE GFA-2-15-60
4: SE GFA-2-15-60	10: SE GFA-2-15-30
[b] 5: KB 4-4-20-30	11: SE GFA-2-10-60
6: SE GFA-2-15-60	

[a] SE GFA–2–30-90: SE = screw element; GFA = forward flight; 2 = double flight; 30 = flight length, 90 = total element length (all in *mm*).

[b] KB 4-4-20-30: KB = kneading block; 4 = number of lobes; 4 = length of each lobe; 20 = pitch of lobes in degrees; 30 = total element length (all in *mm*).

[c] KB 5-2-20-45 R: R = reverse.

Figure 1. Overall barrel temperature and screw configuration profile of laboratory twin-screw extruder.

Table 2. Simple Correlation Coefficients (*r*) between Hardness-Associated Properties and Content of Endosperm Protein, Total Zein, and Zein Subclasses[a]

quality measurement	protein	total zein	α-zein	β-zein	γ-zein
test wt (kg/hL)	0.925**	0.917**	0.882* (0.653*)[a]	–0.460 (–0.667*)	0.581 (0.381)
air pycnometer density (g/cm^3)	0.841*	0.669	0.662 (0.338)	–0.396 (–0.577*)	0.353 (0.154)
TADD (%)	0.951*	0.894*	0.861* (0.577*)	–0.470 (–0.667*)	0.584 (0.364)
time to grind in SHT test (s)	0.187	0.228	0.418 (0.570)	–0.875* (–0.714**)	–0.443 (–0.544)
absorbance at 860 nm in NIT	–0.900*	–0.879*	–0.928** (–0.753**)	0.743 (0.853**)	–0.291 (–0.053)

[a] Simple correlation coefficents between hardness-associated properties and the percentage of zein subclass at constant protein content. *, significance at $p < 0.05$; **, significance at $p < 0.01$.

across the standard curve was seen with an r^2 value of 0.98 between the concentration and the observances at 595 nm.

Free Sulfhydryl, Disulfide, and Total Cysteine Contents. For free sulfhydryl, disulfide, and total cysteine determination, the methods described in Thannhauser et al. (*29*) and Chan and Wasserman (*30*) were used.

Starch Characterization. Megazyme assay kits (Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland) were used for the determination of total starch (AACC methods 77-12) and amylose content. Damaged starch granules (degree of gelatinization) in corn grits and extrudates were estimated by treating the samples with two polysaccharide hydrolyzing enzymes, α-amylase and amyloglucosidase, by using a Megazyme assay kit (Megazyme International Ireland Ltd.). Averages of three replicated measurements were reported. For high-performance size exclusion chromatography (HPSEC), ground grits and extrudates (1% w/v, dry matter) were suspended in 90% dimethyl sulfoxide (DMSO). The sample suspension was placed in a boiling water bath for 1 h with frequent vortexing. The suspension was centrifuged at 3200 rpm for 30 min at room temperature and filtered with a 1.2 μm syringe nylon filter. The filtrate was injected into the HPSEC system (Waters Associates, Milford, MA), which consists of S-805/S, S-804/S, and S-803/S columns (Showa Denko, Tokyo, Japan) in series. The columns were connected to a Waters Associates model 410 refractive index detector. Deionized−distilled water (mobile phase) was used at a flow rate of 1 mL/min at ambient temperature. The column and the detector cell were maintained at 70 and 50 °C, respectively. Data were collected using the Millenium32 software version 3.05.01 (Waters Corp., Milford, MA).

Statistical Analyses. Pearson's simple and partial coefficients with probability of a zero coefficient were determined for statistical correlations. Means differences among maize hybrids and between harder and softer grits and extrudates were evaluated by analysis of variance (one- or two-way ANOVA). Data were analyzed using SAS software (*31*).

RESULTS AND DISCUSSION

Protein Properties of Grits. Maize kernel hardness-associated properties were highly correlated with the content of total endosperm protein, total zeins, α-zeins, and β-zeins, but not with γ- zeins (**Table 2**). These results imply that zein subclasses are to some extent associated with maize kernel hardness as reported in previous studies (*5*, *6*, *9*).

Each maize hybrid displayed a unique RP-HPLC chromatogram as well as zein subclass composition (**Table 3**). In chromatograms of zeins solubilized in 60% *tert*-butyl alcohol plus 2% β-ME, harder grits tended to have a greater amount of earlier eluting (≈21−25 min) zeins (Z1) and to contain a lower content of second eluting (25−29 min) zeins (Z2). Z1 peak corresponds to γ-zeins, and Z2 peak β-zeins, respectively (see **Figure 2** for location of peaks in HPLC chromatograms) (*27*). The ratio of Z1/(Z1 + Z2) was highly correlated with maize kernel hardness-associated properties including TADD ($r = 0.78$, $p < 0.01$) and test weight ($r = 0.77$, $p < 0.01$). Because

Table 3. Amounts of Zein Subclasses Eluting at Different Times in RP-HPLC Chromatograms for Six Maize Grit Samples[a,b]

sample	Z1 (mg)	Z2 (mg)	Z1 + Z2 (mg)	Z1/ (Z1 + Z2)	Zα1 + Zα2 (mg)
HARD1	0.32	0.61	0.93	0.34	6.70
HARD2	0.48	0.81	1.29	0.37	7.06
HARD3	0.54	0.71	1.25	0.43	7.08
SOFT1	0.36	0.74	1.10	0.33	6.51
SOFT2	0.47	0.94	1.41	0.33	5.40
SOFT3	0.31	0.88	1.19	0.26	5.54

[a] Data shown are an average of duplicate measurements. [b] Z1, Z2, and Zα peaks in RP-HPLC chromatogram correspond to γ-, β-, and α-zeins, respectively.

the Z1 peak was extracted with reducing solution and known to be mainly γ-zeins having high levels of cysteine, disulfide formation should be involved in maize kernel hardness, which supports previous works on maize kernel hardness (*5*, *6*). However, HARD1 grits contained much less Z1 than the other harder grits but a similar amount of Zα1 + Zα2 (mainly α-zeins), which indicates that α-zeins and their chemical interactions contribute to maize endosperm texture more than other zein subclasses.

Protein Properties of Extrudates. Protein solubility significantly reduced in response to the extrusion process due to protein denaturation and aggregation as evident in size exclusion chromatography and RP-HPLC results. The fragmentation of zein polymers increased with screw speeds, indicated by the smaller area in the size exclusion chromatograms (**Figure 3**). Softer grit extrudates experienced greater zein molecular fragmentation than harder grit extrudates. Thus, the differences in extrudate properties due to different screw speed and maize endosperm texture seemed to be partially explained by the extent of zein protein fragmentation. The RP-HPLC chromatograms of the samples extracted with reducing solution showed minor quantitative changes; however, samples extracted with nonreducing solution showed a much greater degree of change. To quantify this, the chromatograms of nonreduced samples (mainly α-zeins) were divided into two areas, area 1 (Zα1), eluting between 37 and 41 min, and area 2 (Zα2), eluting between 41

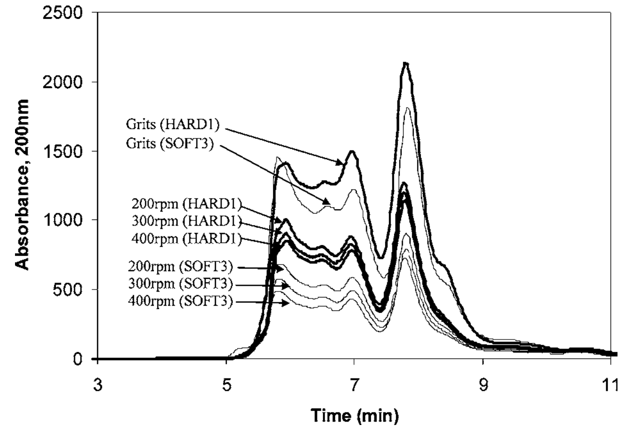

Figure 3. Size exclusion chromatograms of zein proteins in HARD1 and SOFT3 grits and extrudates at different screw speeds. Bold lines represent HARD1 grits and extrudates.

and 48 min (**Figure 2**). ANOVA of the peak areas in chromatograms showed that there were significant differences in the ratio of reduced extrudates to reduced grits among sample grits ($p < 0.01$) and between harder and softer grits ($p < 0.01$). The ratios were always less in chromatograms of reduced harder grit extrudates than in reduced softer grit extrudates (**Table 4**), indicating less solubility of reduced zeins, possibly from greater protein aggregation in harder grits than softer grits during extrusion process. With nonreduced grits and extrudates, the ratio of extrudates to grits was significantly different among sample grits ($p < 0.01$), but no significant difference was found between harder and softer grits ($p = 0.679$). Interestingly, the more hydrophobic nonreduced zein fractions appeared to be more affected by the extrusion process because the Zα2 peaks markedly changed and decreased after extrusion compared with the Zα1 peaks (**Figure 2** and **Table 4**). It is speculated that protein bodies are disrupted followed by hydrophobic α-zeins being released and dispersed during the extrusion process as described in Batterman-Azcona et al. (*32*). The decrease in protein solubility is believed to result from the combined effect of hydrophobic interaction and disulfide bond formation.

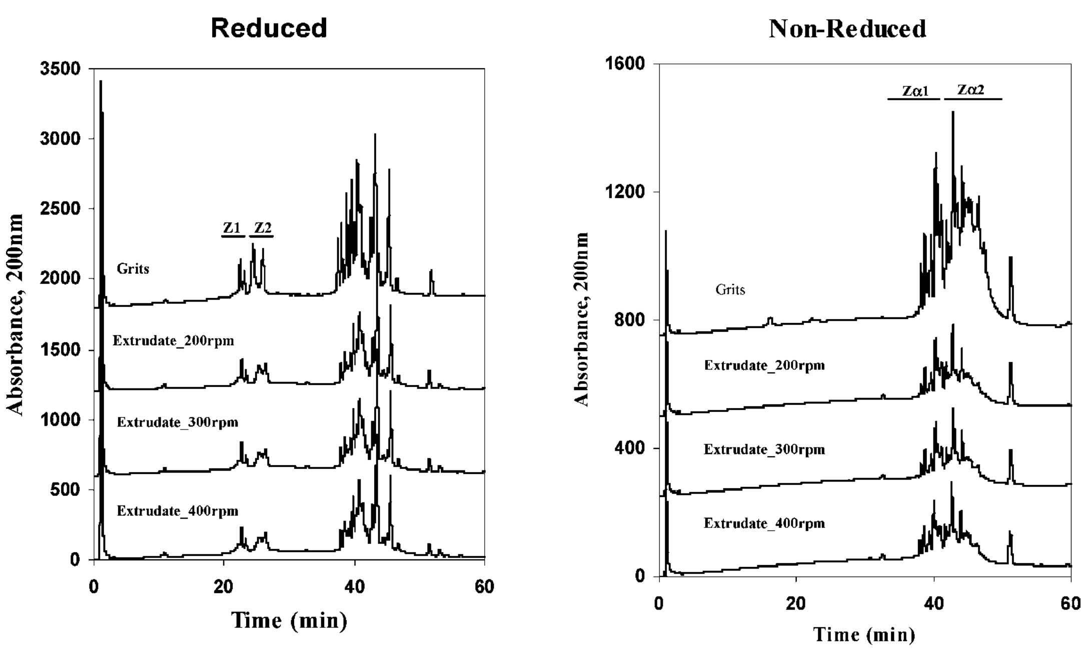

Figure 2. RP-HPLC chromatograms of alcohol-extractable proteins in HARD2 grits and extrudates at different screw speeds using 60% *tert*-butyl alcohol with or without reducing agent (β-ME).

Table 4. Ratio of Extrudates to Grits Based on Amounts of α-Zeins Extracted Using Reducing and Nonreducing Solutions[a]

sample	Rex/Rgr[b]	NRex/NRgr (Zα1)[c]	NRex/NRgr (Zα2)[d]
HARD1	0.665 abc	0.375 a	0.287 b
HARD2	0.632 ab	0.468 c	0.360 c
HARD3	0.630 a	0.300 a	0.195 a
SOFT1	0.718 d	0.342 ab	0.255 b
SOFT2	0.668 bc	0.482 c	0.298 b
SOFT3	0.673 c	0.388 a	0.260 b

[a] Data followed by the same letters are not significantly different at $p < 0.05$. [b] Rex/Rgr, ratio of the reduced extrudates to reduced grits for α-zeins. [c] NRex/NRgr (Zα1), ratio of nonreduced extrudates to nonreduced grits for α-zeins eluting between 37 and 41 min. [d] NRex/NRgr (Zα2), ratio of nonreduced extrudates to nonreduced grits for α-zeins eluting between 41 and 48 min.

Table 5. Free Sulfhydryl and Disulfide Contents per Milligram of Protein and in Total Protein in Maize Grits and Extrudates[a]

		free sulfhydryl		disulfide	
sample	screw speed (rpm)	mg of protein[b]	total protein[c]	mg of protein	total protein
HARD1	grit	41.1 a	358.7 a	28.5 cdef	248.9 defg
	200	32.5 bcd	275.8 bcdef	23.4 hij	198.5 ij
	300	33.2 bc	287.4 bc	24.5 ghij	211.4 hij
	400	31.5 cd	277.9 bcde	21.8 j	197.8 j
HARD 2	grit	30.0 cdef	285.5bc	31.4 bcde	298.3 abc
	200	25.9 g	234.1 hijk	29.4 cdef	265.2 cd
	300	27.2 efg	251.8 efgh	27.9 defg	258.3 def
	400	24.8 g	233.7 hijk	28.9 cdef	272.4 bcd
HARD3	grit	31.8 cd	283.6 bcd	33.7 ab	300.1 ab
	200	30.4 cde	265.7 cdefg	27.5 efg	240.9 defgh
	300	24.2 g	212.8 jk	22.4 ij	198.9 ij
	400	26.5 gf	234.5 hijk	28.4 cdef	250.8 defg
SOFT1	grit	33.1 bcd	281.8 bcde	31.9 bc	271.2 bcd
	200	25.8 g	217.2 ijk	26.5 fghi	222.7 ghij
	300	25.1 g	213.1 jk	27.1 fgh	229.6 efghij
	400	24.5 g	215.7 ijk	27.8 defg	245.2 defg
SOFT2	grit	36.0 b	297.5 b	37.3 a	307.8 a
	200	29.6 def	242.3 ghij	32.0 bc	261.9 de
	300	29.9 cdef	246.5 fghi	31.4 bcd	259.6 def
	400	30.2 cde	253.3 defgh	31.3 bcde	261.9 de
SOFT3	grit	32.3 cd	252.9 defgh	33.5 ab	262.8 de
	200	27.4 efg	210.1 k	30.0 bcdef	230.0 efghij
	300	26.9 efg	211.6 jk	29.5 cdef	231.7 efghi
	400	27.3 efg	216.5 ijk	28.7 cdef	227.3 fghij

[a] Data followed by the same letters are not significantly different at $p < 0.05$. [b] Values are mean of duplicate measurements and reported in nmol/mg of protein. [c] Values are calculated by total protein content (mg) × contents (nmol) per mg of protein, reported in nmol.

However, the results in **Figure 2** for the nonreduced sample show that specific hydrophobic groups of proteins (i.e., those in Zα2) may have a crucial role in the extrusion of maize.

Table 5 shows free sulfhydryl and disulfide contents per milligram of protein and in total protein for maize grits and extrudates. Free sulfhydryl contents of extrudates were lower than those of their corresponding grits. The decrease in free sulfhydryl content may be attributed to lower protein solubility due to the denaturation and aggregation of protein molecules through disulfide formation and hydrophobic interaction after extrusion. This result is consistent with that of size exclusion chromatography and RP-HPLC, where decreased protein solubility of extrudates was observed. The amounts of disulfide bonds in **Table 5** are roughly proportional to those of β- and γ-zeins (**Table 3**), which contain high levels of cysteine residues. The SOFT2, HARD2, and SOFT3 hybrids yielded extrudates

Table 6. Total Starch and Amylose Contents, Ratio of Amylose and Amylopectin, and Starch Damage of Maize Grit Samples[a]

sample	starch (%, wb)	amylose (%)	amylose/ amylopectin	starch damage (%)
HARD1	74.72 ± 1.08	25.25 ± 1.96	0.338 ± 0.036	11.17 ± 0.18
HARD2	75.13 ± 0.81	25.51 ± 0.92	0.343 ± 0.017	10.16 ± 0.50
HARD3	74.96 ± 0.89	25.39 ± 1.04	0.341 ± 0.019	10.13 ± 0.39
SOFT1	75.92 ± 1.02	24.82 ± 0.69	0.330 ± 0.012	10.07 ± 0.29
SOFT2	76.05 ± 0.81	24.10 ± 0.57	0.318 ± 0.010	7.42 ± 0.14
SOFT3	76.23 ± 0.92	24.19 ± 1.60	0.319 ± 0.028	7.19 ± 0.18

[a] Values shown are mean ± standard deviation of four or three (starch damage) replicates.

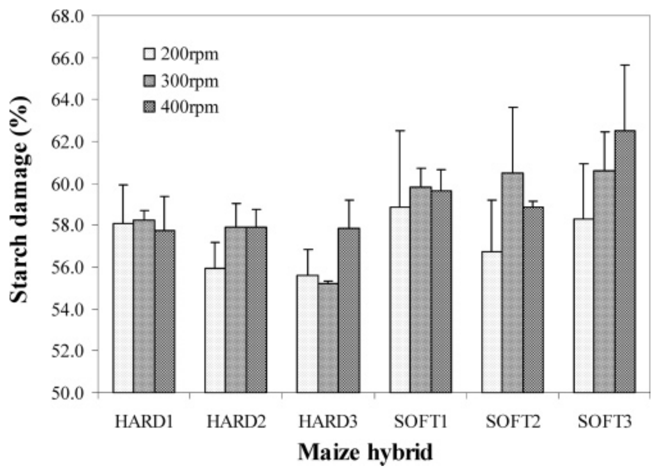

Figure 4. Degree of starch damage in harder and softer grit extrudates at different screw speeds. Error bars represent standard deviation.

with greater amounts of disulfide bonds, which is consistent with greater amounts of β- and γ-zeins in their grits. On the contrary, the HARD1 hybrid that contains the lowest amounts of β- and γ-zeins showed the least disulfide bonds among grit extrudates. HARD1 and SOFT1 grits have similar levels of total protein content, but different levels of disulfide bond and zein subclass composition. The level of disulfide bonds was higher in SOFT1 grits (**Table 5**), whereas the α-zein content was higher in HARD1 grits (**Table 3**). These results suggest that extrudate differences may be closely associated with zein subclass composition, particularly, specific hydrophobic protein groups (mainly α-zeins) as seen in RP-HPLC separation.

Starch Properties of Grits. Total starch contents of softer grits were greater than those of harder grits, whereas softer grits contained less amylose in starch granules than harder grits (**Table 6**). Maize kernel hardness-associated properties measured in this study were highly associated with starch and amylose contents and the ratio of amylose and amylopectin. Amylose content was highly and significantly correlated to test weight ($r = 0.82$, $p < 0.01$), air comparison pycnometer density ($r = 0.71$, $p < 0.01$), and TADD ($r = 0.89$, $p < 0.01$). This finding is consistent with previous studies (*4*, *18*).

Starch damage was higher in harder grits (**Table 6**) and lower in harder grit extrudates (**Figure 4**). Starch granules are protected by a protein matrix against mechanical shear strain and heat imposed on starch granules (*33*), resulting in the partial or entire preservation of crystalline structure of starch molecules. Starch damage increased with screw speed, but no difference was found between 300 and 400 rpm ($p < 0.05$). Increased or decreased starch damage with increasing screw speed can be ascribed to the relatively predominant role of either mechanical shear stress or residence time (*34*). In this study, the effect of mechanical

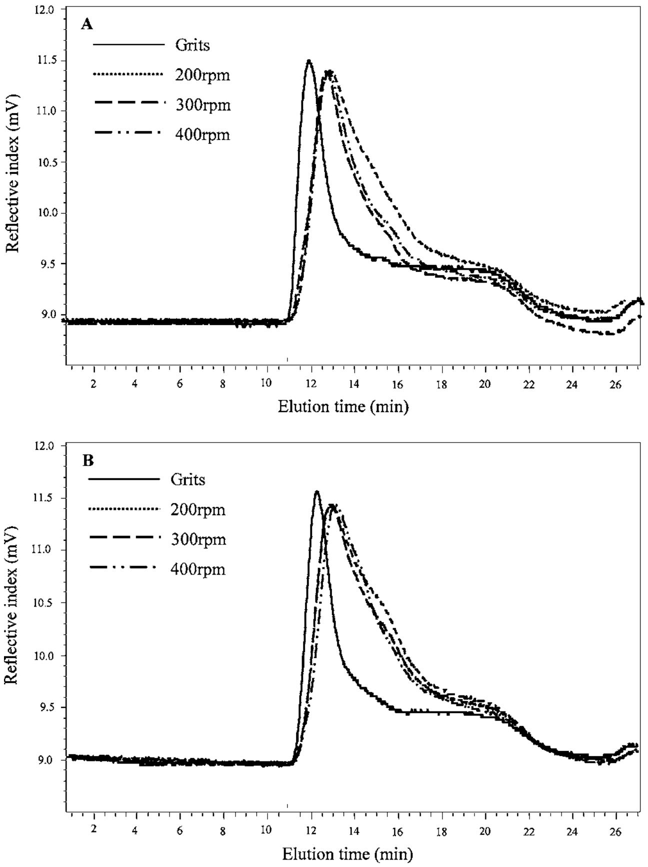

Figure 5. HPSEC elution profile of HARD1 (**A**) and SOFT3 (**B**) grits and extrudates at different screw speeds.

shear stress was more dominant than the thermal effect because barrel temperatures were similar for all treatments. Damaged starch granules are more susceptible to thermal influence, reducing intrinsic viscosity and thus increasing expansion ratio (*35*).

HPSEC chromatograms (**Figure 5**) of extrudates indicated that the large amylopectin molecules were significantly reduced, increasing the fraction of the intermediate molecules between amylopectin and amylose faction. Amylopectin molecules of softer grit extrudates appeared to be more fragmented compared to harder grit extrudates, likely due to the protective effect of protein on starch granules in the harder grits. The extent of starch fragmentation is thought to be linearly proportional to starch gelatinization (*36*, *37*). Nondisrupted starch molecules were reported to not be susceptible to fragmentation by mechanical shear stress (*38*). The extent of starch granule and molecular fragmentation within a single hybrid was not in proportional to screw speed, but depended upon the relative importance of mechanical shear stress (SME) and residence time at constant barrel temperatures (*39*).

Extrudate Properties. The significance of the main effects and their interaction for specific mechanical energy and selected extrudate properties are shown in **Table 7**. Two main effects, maize hardness and screw speed, influenced most of the extrudate properties. Simple correlation coefficients and their significance for extrudate properties and extrusion-processing parameters are shown in **Table 8**.

The specific mechanical energy required to produce an extrudate was lower in harder grits than in softer grits (**Figure 6A**) and increased with screw speed (**Figure 7A**). A high correlation occurred between specific mechanical energy and expansion ratio ($r = 0.86$, $p < 0.01$). Robutti et al. (*9*) reported a similar result using flint, intermediate, and dent-type maize hybrids. In a previous study, the decreased specific mechanical energy with increasing screw speed was observed when increased screw speed caused a significant decrease in melt viscosity (*23*). The higher specific mechanical energy of softer grit extrudates is thought to lead to more damage and breakdown of starch molecules as described in previous studies (*13*, *39*, *40*).

The expansion ratio responded significantly to maize hardness and screw speed. Softer grit extrudates displayed a significantly ($p < 0.01$) higher expansion ratio than harder grit extrudates (**Table 7**). SOFT3, which has the lowest kernel hardness, exhibited the greatest expansion among grit extrudates, whereas HARD1, one of the harder maize samples, exhibited the least

Table 7. Statistical Significance (*p* Value) for the Effects of Maize Kernel Hardness and Screw Speed on Specific Mechanical Energy, Expansion Ratio, Water Absorption Index, Water Solubility Index, Oil Absorption Capacity, and Breaking Stress

	specific mechanical energy (kJ/kg)	expansion ratio	water absorption index (g of gel weight/g of dry sample)	water solubility index (% dry matter)	oil absorption capacity (g of oil/g of dry sample)	breaking stress (kPa)
hardness (H)	0.048	<0.01	0.091	<0.01	<0.01	<0.01
screw speed (S)	<0.01	<0.01	0.095	<0.01	<0.01	<0.01
H × S	0.575	0.288	0.949	0.547	0.056	0.434

Table 8. Simple Correlation Coefficients (*r*) and Their Significance among Selected Extrudate Properties and Extrusion Processing Conditions[a]

	RPM	Die_T	Die_P	ER	PD	SME	WAI	WSI	OAC
Die_T	0.44								
Die_P	−0.61***	−0.13							
ER	0.84***	0.43	−0.36						
PD	−0.87**	−0.45	0.36	−0.98***					
SME	0.90***	0.54*	−0.36	0.86***	−0.85***				
WAI	0.41	−0.19	0.04	0.62***	−0.61***	0.40			
WSI	0.82***	0.55**	−0.24	0.94***	−0.95***	0.86***	0.55*		
OAC	0.82***	0.47*	−0.25	0.78***	−0.81***	0.88***	0.38	−0.89***	
BS	−0.85***	−0.42	0.41	−0.91***	0.92***	−0.85***	−0.52*	−0.87***	−0.84***

[a] RPM, screw speed (rpm); Die_T, die temperature (°C); Die_P, die pressure (psig); ER, expansion ratio; PD, piece density (g/cm^3); SME, specific mechanical energy (kJ/kg); WAI, water absorption index (g of gel weight/g of dry sample); WSI, water solubility index (% dry matter); OAC, oil absorption capacity (g of oil/g of dry sample); BS, breaking stress (kPa). *, significance at $p < 0.05$; **, significance at $p < 0.01$; ***, significance at $p < 0.001$.

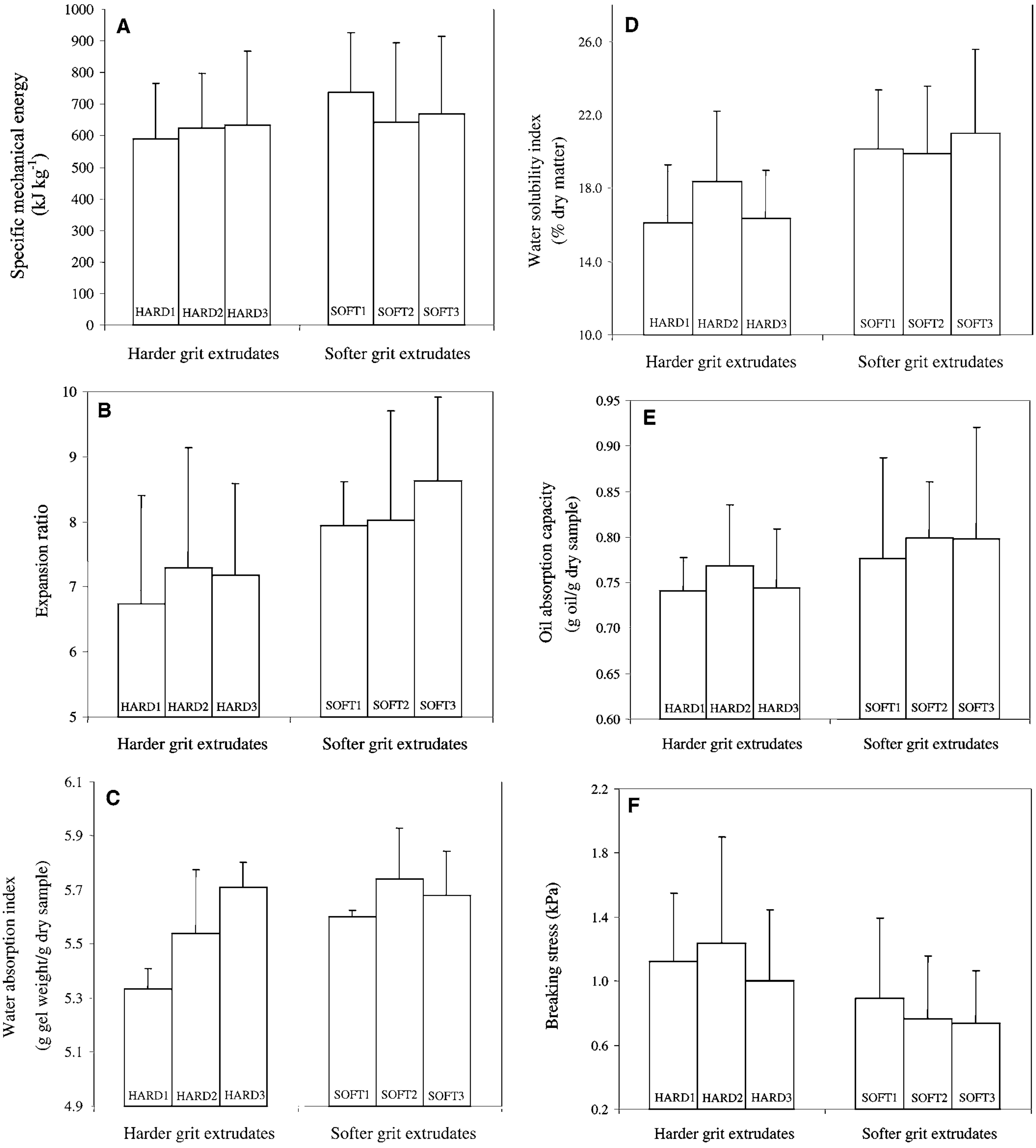

Figure 6. Effect of maize hardness on specific mechanical energy (kJ kg^{-1}) and extrudate properties: (**A**) specific mechanical energy; (**B**) expansion ratio; (**C**) water absorption index; (**D**) water solubility index; (**E**) oil absorption capacity; (**F**) breaking stress. Error bars represent standard deviation.

expansion ratio (**Figure 6B**). The expansion ratio increased as screw speed increased (**Figure 7B**) and was highly and negatively correlated with piece density ($r = -0.98$, $p < 0.01$). An increasing screw speed results in greater specific mechanical energy, which leads to a higher die temperature (driving force) and lower melt viscosity (resisting force), resulting in a higher expansion ratio. The quantity and quality of ingredients, including lipids (*8*), protein (*21*, *32*), and starch (*3*), also determine the expansion ratio. Grit samples were extruded under constant extrusion processing conditions including feed rate, feed moisture content, and barrel temperature. As a result, starch and protein macromolecules and their interactions within an extruder may become important factors affecting the expansion ratio of the extrudates. It has been reported that the expansion ratio of starch extrudates increased in proportion to starch and amylose content (*3*, *12*). Thus, a lower amylose content in softer grit extrudates is unlikely to explain their higher expansion ratio in this study. The expansion ratio of extrudates appeared to increase with increasing molecular fragmentation and thus decreasing intrinsic viscosity at lower moisture content (18%). This is in

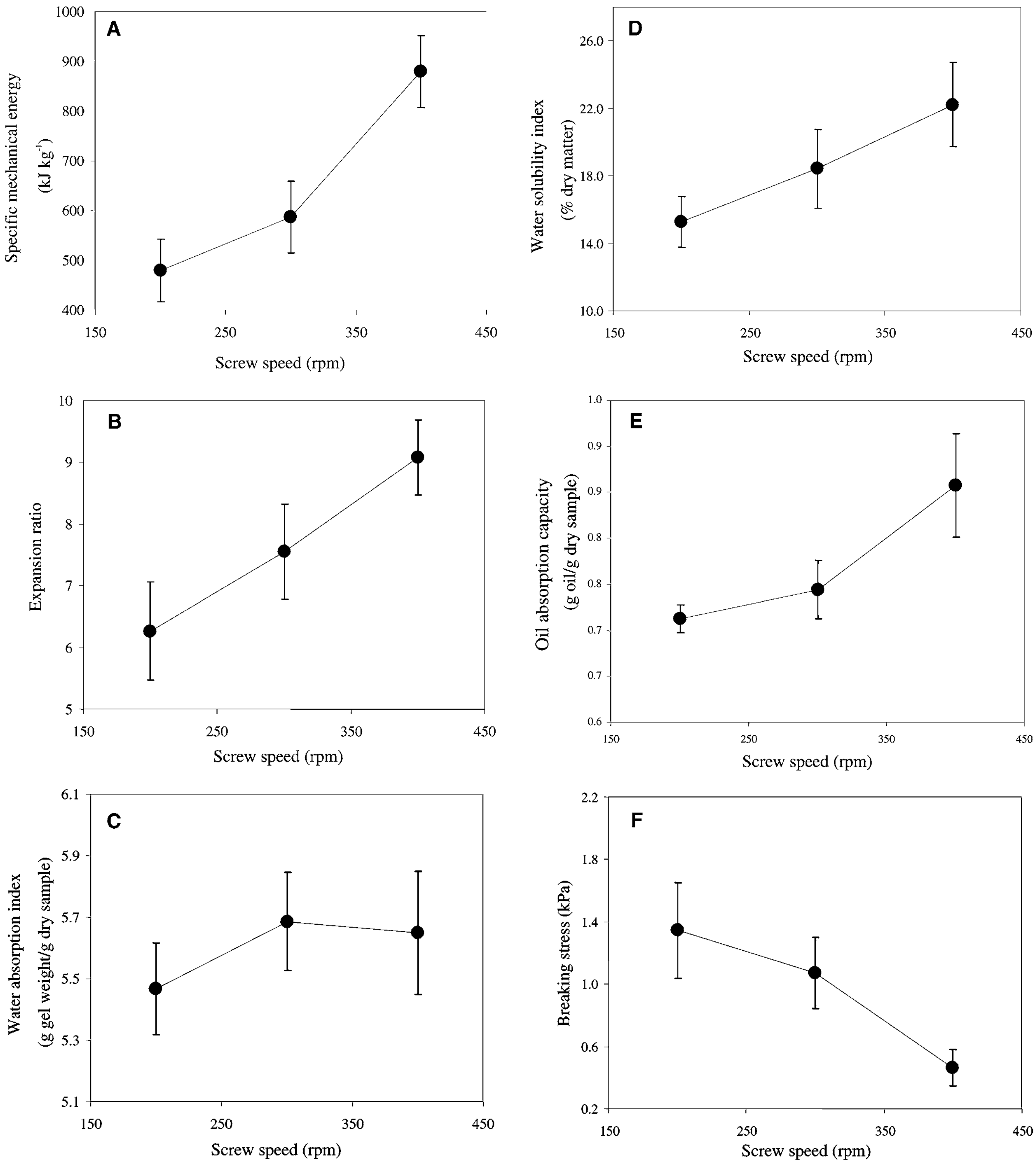

Figure 7. Effect of extruder screw speed on specific mechanical energy (kJ kg^{-1}) and extrudate properties: (**A**) specific mechanical energy; (**B**) expansion ratio; (**C**) water absorption index; (**D**) water solubility index; (**E**) oil absorption capacity; (**F**) breaking stress. Error bars represent standard deviation.

agreement with the findings of Bruemmer et al. (*40*). However, this explains only in part proportional increases in expansion ratio of extrudates with increasing screw speed. ANOVA results showed that the ratios of α-zeins extracted from extrudates to grits (under reducing conditions) were significantly lower in harder grits than in softer grits ($p < 0.05$), indicating that zein proteins from softer grits aggregated less than those from harder grits. It is generally accepted that increasing the amount of proteins tends to reduce the die swell of starch polymers (*8*). Disulfide bonds and hydrophobic interactions that create aggregates and protein fibrils are prevalent in proteins of extrudates (*32*). Disulfide bond-linked protein aggregates in extrudates have been regarded as a structural organizer confining starch molecules that are smaller than those in native starch (*3*). Therefore, higher expansion in softer grit extrudates and gradual increase in expansion with screw speed are thought to result from the combination of less protein aggregation, larger protein fragmentation, higher starch content, and larger starch gelatinization, and molecular fragmentation.

The water absorption index did not respond significantly ($p < 0.05$) to hardness or screw speed. However, softer grit extrudates tended to display a higher water absorption index ($p = 0.091$) compared to extrudate from harder grits (**Figure 6C**). Also, an increasing screw speed above 300 rpm resulted in a slightly decreased water absorption index (**Figure 7C**). Particularly, hybrid HARD1 had a significantly lower water absorption index compared with the others. This could be ascribed to higher remnants of crystalline structure as a result of less starch damage and molecular fragmentation, resulting in inaccessibility of compact structure to water (*39*, *41*). In this study, extrudates with higher water absorption index contain greater amounts of hydrophilic β- and γ-zeins but less hydrophobic α-zeins. Therefore, in part, the availability of hydrophobic zeins is also likely to explain the water absorption index of final extrudates (*21*, *36*, *41*).

The water solubility index was distinctively higher in softer grit extrudates (**Figure 6D**) and increased as screw speed increased (**Figure 7D**). A significantly positive correlation between water solubility index and expansion ratio was found ($r = 0.94$, $p < 0.01$). This indicates that the larger expansion ratio provides a larger surface area for water to interact with starch and other soluble components (*23*). The increased water solubility index is partially attributed to increased molecular solubility and dispersibility of amylose and amylopectin molecules, as evidenced by the positive correlation between water absorption and the water solubility index. This suggests that oligosaccharides resulting from starch fragmentation are not a main contributor to water solubility (*39*).

Harder grit extrudates showed significantly lower oil absorption capacity than softer girt extrudates (**Figure 6E**). Oil absorption capacity was more pronounced at higher screw speeds (**Figure 7E**) and had a significant and high correlation to expansion ratio ($r = 0.78$, $p < 0.01$) and specific mechanical energy ($r = 0.88$, $p < 0.01$). The oil absorption capacity is influenced by several factors including the amount of nonpolar amino acids, sequence of polypeptides, different conformational features of macromolecules, and starch−protein−lipid bindings (*22*). Contrary to the water absorption index, nonpolar side chains of proteins are believed to bind the hydrophobic chains of fat contributing to higher oil absorption (*25*, *41*). It is surmised that protein and starch molecules in softer grit extrudates are less aggregated and more easily fragmented into smaller molecules on the basis of the results obtained from RP-HPLC, size exclusion chromatography, starch damage, HPSEC, and other extrudate properties. This is likely to make oil molecules more accessible to extrudate structure and, thus, proteins able to bind more oil molecules in softer grit extrudates.

Breaking stress of extrudates responded significantly to maize hardness (**Figure 6F**) and screw speed (**Figure 7F**). Breaking stress was negatively correlated to expansion ratio ($r = -0.91$, $p < 0.01$), due to expanded products having a more brittle structure (*42*). Breaking stress appears to be largely determined by degree of expansion and cell wall strength (*43*). In our study, the breaking stress of harder grit extrudates was higher than that of softer grit extrudates, indicating that the breaking stress of extrudates is partially determined by protein aggregation and fragmentation and starch gelatinization and fragmentation.

In conclusion, harder and softer maize hybrids demonstrated significantly different performances during extrusion process. Protein properties including total protein content, zein subclass composition (mainly hydrophobic α-zeins), and protein molecular fragmentation partially explained the differences between physical kernel hardness and extrusion-processing performance. Total starch content, starch gelatinization, and starch molecular fragmentation also contributed to differences in extrudate properties. However, differences in extrudate properties did not appear to be related to disulfide bond formation and amylose content as previously reported. Rather, differences in grit and extrudate properties should be attributed to the combined effect of protein (mainly zeins) and starch properties reflecting maize endosperm texture. Zein proteins are thought to form aggregates present in the amorphous continuous starch matrix due to their immiscibility as observed by microscopes (*32*, *44*). The effect of simultaneous changes in zein and starch molecules on extrudate properties is not clear. There is a need to extend the study for the effects of other extrusion process variables on extrudate properties.

ABBREVIATIONS USED

β-ME, β-mercaptoethanol; BS, breaking stress; DEN_P, air pycnometer density; Die_T, die temperature; Die_P, die pressure; ER, expansion ratio; HPSEC, high-performance size exclusion chromatography; L, motor load on the drive motor; L_0, motor load with no material in barrel; N, screw speed; $N_{a,}$ actual operating screw speed; N_f, full (rated) screw speed; NIT, near-infrared transmittance; OAC, oil absorption capacity; PD, piece density; P_e, rated power of extruder drive motor; Q_{me}, mechanical energy rate; RP-HPLC, reversed-phase high-performance liquid chromatography; RPM, screw speed; SHT, Stenvert hardness tester; SME, specific mechanical energy; TADD, tangential abrasive dehulling device; TTG, time to grind in SHT; TW, test weight; W860, absorbance at 860 nm in NIT; WAI, water absorption index; WSI, water solubility index; Zα1, α-zeins eluting between 37 and 41 min in RP-HPLC; Zα2, α-zeins eluting between 41 and 48 min in RP-HPLC; Z1, earliest eluting (≈21−25 min) zeins in RP-HPLC; Z2, second eluting (≈25−29 min) zeins in RP-HPLC.

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